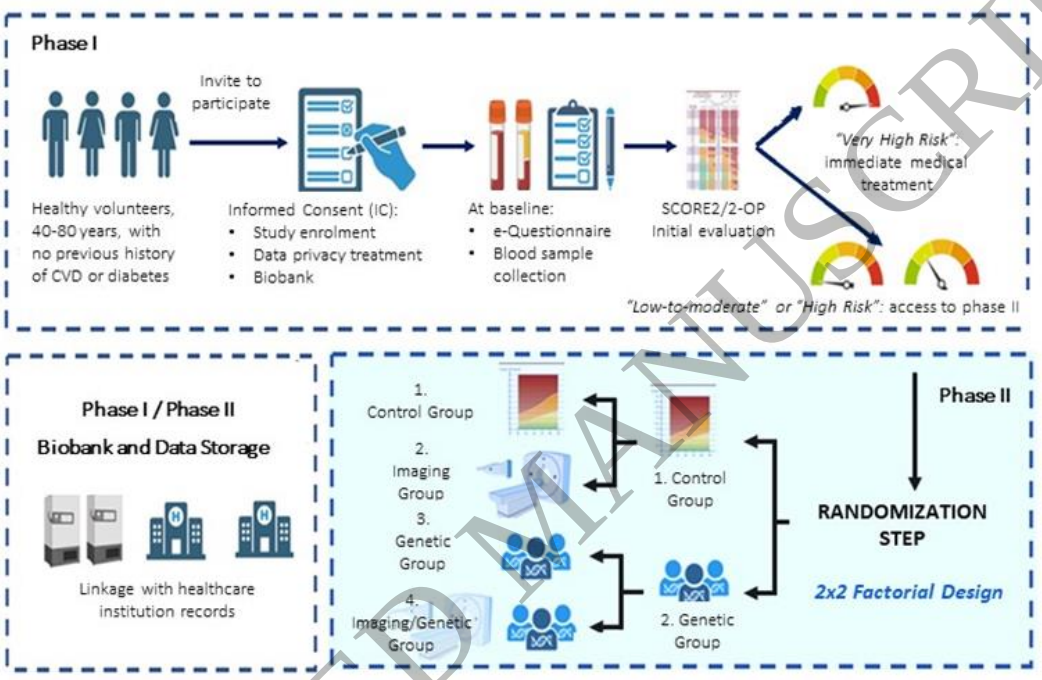
Integrating genetic and imaging information to enhance cardiovascular risk stratification: rationale and design of the CVRISK-IT randomised controlled trial

Emanuele Di Angelantonio[1,2,3,4,5]*, Massimo Piepoli[6]*, Serenella Castelvecchio[7], Chiara Barberio[8], Carmelina Chiarello[8], Ambra Cerri[8], Sara Boveri[8], Rosanna Cardani[9], Federico Ambrogi[8, 10], Sebastian Guelfi[1,8], Damiano Baldassarre[11,12], Gualtiero Colombo[11], Mauro Amato[11], Roberta Baetta[11], Eloisa Arbustini[13], Giovanna Liuzzo[14,15], Stephen Kaptoge[2,3], Anna Severino[14,15], Giuseppe Ferrante[16,17], Maria Teresa La Rovere[18], Gianfranco Parati[19,20], Martino F. Pengo[19,20], Roberto Latini[21], Maria Carla Roncaglioni[21], Marta Rigoni[22,23], Amalia De Curtis[24], Luigi Frati[24],Pietro Ameri[25,26], Paolo G. Camici[27], Massimo Volpe[28], Lorenzo Menicanti[7,8]

[1]Health Data Science Research Centre, Human Technopole, Milan, Italy;
[2]British Heart Foundation Cardiovascular Epidemiology Unit, Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK;
[3]Victor Phillip Dahdaleh Heart and Lung Research Institute, University of Cambridge, Cambridge, UK;
[4]British Heart Foundation Centre of Research Excellence, University of Cambridge, Cambridge, UK;
[5]National Institute for Health and Care Research Blood and Transplant Research Unit in Donor Health and Behaviour, University of Cambridge, Cambridge, UK;
[6]Department of University Cardiology, IRCCS Policlinico San Donato, San Donato Milanese, Milan, Italy;
[7]Department of Cardiac Surgery, IRCCS Policlinico San Donato, San Donato Milanese, Italy;
[8]Scientific Direction, IRCCS Policlinico San Donato, San Donato Milanese, Italy;
[9]BioCor Biobank, IRCCS Policlinico San Donato, San Donato Milanese, Italy;
[10]Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy;
[11]Centro Cardiologico Monzino IRCCS, Milan, Italy;
[12]Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Milan, Italy;
[13] Centre for Inherited Diseases, Department of Research, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy;
[14]Università Cattolica del Sacro Cuore - Campus di Roma, Rome, Italy;
[15]Dipartimento di Scienze Cardiovascolari, Fondazione Policlinico Gemelli IRCCS, Rome, Italy;
[16]Department of Cardiovascular Medicine, IRCCS Humanitas Research Hospital, Rozzano, Italy;
[17]Department of Biomedical Sciences, Humanitas University, Milan, Italy;
[18]Department of Cardiology, Istituti Clinici Scientifici Maugeri IRCCS, Scientific Institute of Montescano, Pavia, Italy;
[19]Department of Cardiology, IRCCS, Istituto Auxologico Italiano, S. Luca Hospital, Milano, Italy;
[20]Department of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy;
[21]Laboratory of Cardiovascular Prevention, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy;
[22]IRCCS Multimedica, Milan, Italy;
[23]Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milano, Italy;
[24]IRCCS Istituto Neurologico Mediterraneo NEUROMED, Pozzilli, Italy;
[25]Cardiovascular Disease Unit, IRCCS Ospedale Policlinico San Martino, Genova, Italy;
[26] Department of Internal Medicine, University of Genova, Genova, Italy;
[27]Università Vita-Salute San Raffaele, Milan, Italy;
[28] IRCCS San Raffaele Rome, Italy.

GRAPHICAL ABSTRACT



ABSTRACT

Introduction Cardiovascular disease (CVD) remains the leading cause of morbidity and mortality worldwide. Current guidelines recommend algorithms to estimate 10-year cardiovascular diseases (CVD) risk in apparently healthy individuals, but their precision remains limited. Whether the integration of imaging and genetic information can improve individual risk stratification remains uncertain.

Methods and Analysis The CVRISK-IT trial is a multicentre, open-label, randomised controlled study designed to enhance CVD prevention through precision-based risk assessment and targeted communication strategies. A total of 30,000 adults aged 40–80 years without previous CVD or diabetes are recruited across Italy. In Phase I, participants undergo a baseline assessment including clinical examination, blood sampling, and calculation of CVD risk. Approximately 12,000 eligible enter Phase II and are randomised equally into four intervention arms: (i) standard care (control), (ii) imaging-based risk refinement (coronary artery calcium score or carotid ultrasound), (iii) polygenic risk score (PRS), or (iv) combination of imaging and PRS. The co-primary endpoints are differences in estimated 10-year CVD risk at 12 months and the incidence of major CVD events, CVD mortality, and all-

cause mortality at 5 years. Secondary endpoints include differences in CVD risk factors, prescription and adherence to preventive drug adherence, and behavioural or psychological measures.

Conclusion By prospectively evaluating the feasibility and impact of incorporating genetic and imaging information in CVD risk stratification, CVRISK-IT will generate robust evidence to inform its integration into routine screening practices and public health policy.

Trial registration: ClinicalTrials.gov Identifier NCT06832644**.**

KEY LEARNING POINTS:

What is already known:

• Conventional CVD risk scores have limited precision in individuals with similar risk factor profiles.

• Imaging and genetic information can improve risk stratification as integrated risk modifiers.

• Evidence from large-scale randomised trials on behavioural or clinical impact of using these methods is lacking.

What this study adds:

• CVRISK-IT is the first large-scale randomised trial which evaluates the impact of incorporating imaging and genetic information into CVD risk stratification, to improve targeting of preventive interventions in primary care.

• It evaluates short- and long-term effects on estimated CVD risk, behaviour, preventive therapy uptake, and outcomes.

• Findings will gain insights on the integration of precision tools into routine CVD prevention and public health policy.

KEYWORDS: *Cardiovascular diseases, Primary prevention, Risk Prediction, SCORE2/SCORE2-OP, Randomized Clinical Trial, Imaging, Polygenic Risk Score, Coronary calcium score (CAC), Carotid B-mode ultrasound*

Background

Cardiovascular disease (CVD) remains the leading cause of death and disability worldwide, accounting for over one-third of all deaths each year.[1] In Europe, despite major advances in prevention and treatment, CVD continues to impose a substantial public health and economic burden.[2,3] Effective primary prevention strategies often involve identifying individuals at increased risk in order to enable timely implementation of targeted lifestyle and pharmacological interventions. The European Society of Cardiology (ESC) currently recommends use of the SCORE2 and SCORE2-Older Persons (SCORE2-OP) algorithms for estimating 10-year CVD risk in apparently healthy adults.[4] These models combine traditional risk factors such as age, sex, blood pressure, smoking, and lipid levels to guide preventive strategies across Europe.[5,6]

However, CVD risk can be substantially different even among people with similar conventional risk factor profiles.[7] To improve risk stratification, the "2021 ESC Guidelines on cardiovascular disease prevention in clinical practice" encourage the use of additional "risk modifiers" including biomarkers such as high-sensitivity C-reactive protein.[4] Recent guidelines and consensus statement have also extended this approach by incorporating both imaging and genetic information as valuable risk modifiers.[8–10] Coronary artery calcium (CAC) scoring is recognised as a practical imaging-based tool to detect subclinical atherosclerosis and to reclassify individuals at borderline or intermediate risk, particularly when treatment decisions are uncertain.[8] When CAC scoring is unavailable or not feasible, carotid ultrasound may be considered as an alternative to detect atherosclerotic disease and refine risk classification around treatment thresholds. [10] Similarly, polygenic risk scores (PRS), which aggregate thousands of genetic variants, can quantify inherited cardiovascular susceptibility, improve risk prediction, and enable earlier preventive action.[9] However, no large-scale randomised studies have examined the impact of integrating imaging and genetic information simultaneously for CVD risk assessment on health-related behaviour, and clinical outcomes. Most studies to date have been observational or limited by small sample sizes, heterogeneous populations, or short follow-up periods.[11–18]

The CVRISK-IT trial was established to address these knowledge gaps by evaluating the short- and long-term effects of integrating imaging and genetic information into CVD risk communication. The study aims to determine whether providing individuals with personalised risk information derived from these modalities enhances risk perception, supports more

tailored lifestyle and treatment recommendations, improves adherence to preventive measures, and ultimately reduces overall CVD risk.

Objectives

Primary Objective

The primary objective of the CVRISK-IT trial is to evaluate the health benefits of incorporating additional CVD risk information, derived from imaging and genetic data, into conventional risk assessment. Specifically, the study aims in the short term to determine whether providing personalised information based on imaging and/or PRS improves changes in estimated 10-year CVD risk, as calculated by the SCORE2/SCORE2-OP algorithms, compared with standard care using conventional risk factors alone. In the longer term, CVRISK-IT will investigate the impact of integrating imaging and genetic information on incidence of major CVD events, CVD mortality, and all-cause mortality.

Secondary Objectives

The secondary objectives are to assess how enhanced risk communication influences modifiable CVD risk factors, and to evaluate changes in preventive drug prescriptions and adherence to recommended therapies. The study will also examine behavioural and psychological outcomes, including changes in risk perception, anxiety, and motivation for lifestyle modification.

Methods

Study design

CVRISK-IT is a multicentre, open-label, randomised controlled trial conducted within the framework of the Italian Cardiology Network (ICN), involving 17 Scientific Institutes for Research, Hospitalisation and Health Care (known by the Italian acronym IRCCS) and their affiliated centres distributed across Italy (**Figure 1** and **eTable 1, Supplementary material**). The study is centrally coordinated by the IRCCS Policlinico San Donato Hospital, with operational support from a dedicated trial management group responsible for protocol implementation, data management, and quality assurance. Oversight is provided by national and institutional ethics committees to ensure compliance with all regulatory and ethical requirements. All study procedures are conducted in accordance with Good Clinical Practice

and the ethical principles of the Declaration of Helsinki. The trial is registered at ClinicalTrials.gov NCT06832644.

The trial includes two phases: Phase I, the establishment of a large national bioresource of adults undergoing CVD risk profiling, and Phase II, a randomised evaluation of different strategies for risk stratification (**Figure 2**).

Participants and recruitment

In Phase I, 30,000 apparently healthy adults aged 40 to 80 years without a history of CVD or diabetes are recruited across Italy through workplace screening programmes, care homes, volunteer organisations, and participating hospitals (**eTable 1, Supplementary material)**. A participant flow diagram following is presented in **eTable 2** of the **Supplementary Material**.[19] Individuals must have sufficient proficiency in Italian to provide informed consent and complete study questionnaires. Exclusion criteria include a previous medical history related to CVD (e.g., heart failure, congenital heart disorder, coronary heart disease) based on medical diagnosis; previous history of type 1 or type 2 diabetes (as considered already at very high risk); medical diagnosis of mental disorders; pregnancy based on self-reported information; oncology patients (based on physician indication); candidates who have previously participated in other interventional trial on CVD prevention [20], and inability or unwillingness to provide biological samples (**eTable 3, Supplementary material**).

Baseline visit procedures

Participants provide informed consent, complete a web-based baseline questionnaire, undergo clinical examination, and provide fasting blood samples for biobanking (**Appendix A**). CVD risk is estimated using SCORE2 or SCORE2-OP algorithms as recommended by the 2021 ESC Guidelines on Cardiovascular Disease Prevention.[4] Individuals identified as having very high CVD risk are referred directly for clinical management and excluded from randomisation, but monitored for long-term incident CVD event. All participants contribute a national biobank and data repository to support future research into chronic disease determinants in Italy.

Randomisation and allocation concealment mechanism

Participants identified as having low-to-moderate or high predicted CVD risk, as defined by the 2021 ESC Guidelines on Cardiovascular Disease Prevention (**Table 1**), are invited to

participate in Phase II. Invitations to Phase II initially follow a first-come, first-served process to ensure balanced representation across participating centres. Subsequently, recruitment will transition to an adaptive allocation framework designed to maintain proportional enrolment across sites and study groups. This pragmatic strategy allows continuous recruitment while maintaining operational feasibility within the multicentre network. Approximately 12,000 individuals will be randomly assigned in equal proportions to one of four study interventions: a "control group" receiving standard SCORE2/SCORE2-OP risk assessment; a "genetic risk group" receiving additional risk information based on PRS; an "imaging risk group" receiving additional risk information derived from imaging-based assessment, including CAC scoring or carotid ultrasound, depending on centre capacity; and a "combined risk group" receiving additional risk information based on PRS and imaging (**Figure 3**). Randomisation is performed centrally using a secure, computerised system employing block randomisation stratified by centre. Block sizes vary randomly, and their sequence and size are concealed from study investigators. Because of the nature of the intervention, participants and clinicians cannot be blinded to group assignment, but data analysts and outcome assessors remain masked to allocation.

Interventions

Risk information is communicated through a standardised in-person or remote medical consultation that combines visual aids and personalised discussion to enhance understanding and motivation. All participants receive structured CVD prevention counselling based on ESC recommendations using a dedicated digital platform, "MyCardioSpace".[21] Recommendations include dietary habits, promotion of physical activity, and smoking cessation[4] and, when indicated, pharmacological prevention, including lipid-lowering and antihypertensive therapy.

Participants allocated to the control arm will receive standard CVD risk estimates derived from SCORE2 or SCORE2-OP, accompanied by the corresponding evidence-based prevention recommendations. Participants in the genetic arm will receive additional risk information derived from a PRS, computed from genome-wide genotyping data and categorised into relative risk strata. Participants in the imaging arm will undergo cardiovascular imaging, either CAC scoring or carotid ultrasound. Imaging findings will be classified according to predefined quantitative thresholds.[21–23] For participants in the genetic and imaging arms, the PRS and/or imaging findings will be used solely for risk reclassification purposes within the overall CVD risk assessment framework. Clinical management recommendations will not be

individualised based on specific PRS percentiles or CAC scores but will instead follow standardised guideline-directed preventive strategies corresponding to the participant's final risk category after reclassification. This approach ensures consistency of preventive advice across study arms while allowing evaluation of the impact of risk modifiers on overall risk stratification (**Appendix B**). Downward reclassification based on imaging or genetic findings is systematically captured for analytical purposes; however, to preserve adherence to current clinical guidelines, it does not lead to de-escalation of recommended preventive strategies within the trial.

Follow-up and outcome measures

Participants are followed up at 12 months after risk communication visit through repeat questionnaires, blood collection, and clinical assessments. Over long-term follow-up, the trial aims to demonstrate whether short-term improvements translate into lower incidence of major cardiovascular events. Long-term outcomes will be tracked through linkage with national registries of hospital admissions and mortality records, in particular CVD events and cause-specific mortality. The co-primary outcomes are the difference in estimated 10-year CVD risk, calculated using SCORE2 or SCORE2-OP, at 12 months and incidence of CVD events (i.e., non-fatal myocardial infarction, acute coronary syndrome, percutaneous transluminal coronary angioplasty [PTCA], coronary artery bypass grafting [CABG], non-fatal stroke, transient ischemic attach [TIA], and new diagnosis or hospitalization for heart failure and atrial fibrillation), CVD mortality, and all-cause mortality at 5-years.

Secondary outcomes include between-group differences in modifiable risk factors such as systolic blood pressure, lipids, smoking status, and body weight (both in isolation and in combination using CVD risk algorithms other than SCORE2;[22] differences in initiation of preventive drug prescriptions and adherence; and behavioural and psychological measures, including perceived risk and motivation for lifestyle change. Longer term follow-up is also planned at 10 years.

Data management and quality assurance

All data are collected electronically through a secure web-based system managed by the central coordinating centre. Data quality is ensured through automated validation checks and regular monitoring visits. Biological samples are processed and stored at the national biobank,

ensuring traceability and long-term preservation (**Appendix C**). Data are pseudonymised and managed in compliance with the General Data Protection Regulation (GDPR) and Italian privacy legislation. Study data are collected and managed using REDCap electronic data capture tools [23,24] hosted on the IT platform of the ICN, developed in collaboration with the Consortium of Bioengineering and Medical Informatics (CBIM).

Sample size and statistical analysis

Sample size calculation

The trial adopts a 2×2 factorial randomised design, allowing simultaneous evaluation of two interventions—genetic and imaging-based CVD risk communication—both independently and in combination. A total of 12,000 participants will be randomised equally across four study groups (control, genetic, imaging, and combined genetic + imaging), corresponding to approximately 3,000 individuals per arm (**Appendix D**). Sample size determination was based on simulation analyses exploring a range of plausible event rates for the CVD incidence co-primary outcome in the control group (2-10%) and assuming relative risks of 0.85 for each intervention. Interaction effects between the two interventions were modelled under different scenarios, including absence of interaction, synergistic, and antagonistic effects. For each condition, data were simulated for 1,000 repetitions and analysed using logistic regression models incorporating the main and interaction effects. These simulations confirmed that a total enrolment of 12,000 participants provides at least 80% power at a two-sided significance level of 0.05 to detect clinically meaningful differences in the primary endpoint across intervention groups, while accommodating potential attrition. To ensure that 12,000 participants are randomised while maintaining balanced representation across centres, Phase I enrolment is set at 30,000 individuals.

Approximately 30% of screened participants are expected to fall into the very-high-risk category by SCORE2/SCORE2-OP and thus be excluded from randomisation. Of the remaining participants, an additional 10–20% are anticipated to decline genetic or imaging procedures, withdraw consent, or face logistical exclusions. This approach is expected to yield approximately 15,000–18,000 eligible participants, ensuring a sufficient number of individuals for Phase II. The analyses were performed using Stata software (StataCorp, College Station, TX).

Statistical analysis

All data will undergo initial analysis in accordance with recommendations from the STRATOS Initiative.[25] This process will include metadata setup, systematic data cleaning, quality screening, and descriptive reporting before formal hypothesis testing. Frequency distributions will be inspected to identify missing data, outliers, and implausible values. Continuous variables will be summarised as mean ± SD or median (interquartile range), depending on distribution; categorical variables will be presented as frequencies and percentages.

The primary analysis will follow the intention-to-treat principle. Frequency of missing outcome data will be compared across intervention groups. Participants with missing follow-up data will be excluded from the primary model (complete case analysis), with sensitivity analyses performed to assess the robustness of findings to missingness. Missing data will primarily be handled by complete-case analysis for the primary model. Multiple imputation will be conducted as part of ancillary sensitivity analyses to assess robustness under missing-at-random assumptions. The effect of the study group (control, genetic, imaging, or combined) on the 12-month differences in estimated CVD risk (SCORE2 or SCORE2-OP) will be assessed using a two-way analysis of covariance (ANCOVA) model, incorporating the main effects of genetic and imaging information and their interaction and adjusting for centre and baseline assessments of the analysed outcomes.

Where a significant interaction is observed, pairwise comparisons will be estimated as follows: (i) genetic + imaging vs. genetic only — to assess the incremental impact of imaging information; (ii) genetic + imaging vs. Imaging only — to assess the incremental impact of genetic information; (iii) genetic + imaging vs. control — to assess the combined effect relative to standard care. If no interaction is detected, main effects will be reported separately for genetic and imaging interventions. Subgroup analyses will also be performed by age, sex, and baseline risk to assess potential effect modification by incorporating relevant interaction terms with interventions. Significant interactions ($p < 0.05$) will prompt stratified reporting by subgroup categories defined by median and quartile cut-offs. Exploratory analyses will compare CAC- versus carotid ultrasound-based reclassification. When long-term follow-up data will become available, time-to-event analyses for CVD events and mortality will be conducted using Kaplan–Meier curves and Cox proportional hazards models. The proportional hazards assumption will be verified using Schoenfeld residuals and formal test assessing interaction of interventions with follow up time. Competing risks will be addressed using Fine–Gray subdistribution hazard models, with Gray's test applied for group comparisons of

cumulative incidence functions. Secondary analyses will similarly evaluate differences in individual risk factors (blood pressure, lipid profile, smoking, body weight), adherence to preventive medication, and psychological or behavioural outcomes.

All analyses will be performed using SAS 9.4 (SAS Institute, Cary, NC) and R software (R Foundation for Statistical Computing, Vienna, Austria). Statistical significance will be set at two-sided $p \leq 0.05$ for assessment of intervention main effects and $p \leq 0.01$ for subgroup analyses.

Ethical considerations

The CVRISK-IT trial has received ethical approval from the Central Ethics Committee and all participating IRCCS institutions. Written informed consent is obtained for both study participation and biobanking in accordance with EU eIDAS and GDPR standards. Oversight is provided by a Trial Steering Committee responsible for scientific and ethical integrity, a Trial Management Group for operational conduct, and an Independent Data Monitoring Committee that regularly reviews recruitment progress, safety data, and study performance.

Given the non-invasive nature of the study, risks to participants are minimal. Potential harms are limited to minor discomfort or anxiety associated with risk disclosure, venipuncture, and imaging procedures. Radiation exposure from CAC scanning will be kept as low as reasonably achievable, following national safety standards. Any adverse or unintended effects related to study participation will be recorded and reviewed by the Independent Data Monitoring Committee (IDMC).

Results

Based on the trial's design and planned co-primary and secondary outcomes, several results can be anticipated. In the short-term (12 months), it is expected that providing additional personalised information derived from imaging and/or polygenic risk scores may lead to reductions in estimated 10-year CVD risk, compared with standard SCORE2/SCORE2-OP–based assessment alone. Participants receiving imaging or genetic risk information—particularly those in the combined arm—might show an improved modification of key risk factors, such as systolic blood pressure, LDL-cholesterol levels, smoking prevalence, and/or body weight.

At the behavioural level, personalised CVD risk communication is expected to positively influence participants' risk perception, motivation for lifestyle change, and engagement with preventive strategies. The digital support platform will aim to further strengthen and enhance these behavioural effects across all intervention groups.

Over long-term follow-up (5 years), the trial aims to demonstrate whether these short-term improvements translate into lower incidence of major cardiovascular events, cardiovascular mortality, and all-cause mortality, especially for the combined imaging + polygenic risk arm.

Overall, the findings are anticipated to provide robust evidence on the feasibility and potential clinical utility of integrating imaging and genetic risk modifiers into routine cardiovascular prevention strategies in apparently healthy adults.

Discussion

Despite major advances in prevention and treatment, population ageing, unhealthy lifestyle habits, and persistent inequalities continue to sustain the CVD burden. Current prevention strategies rely on established algorithms such as SCORE2 and SCORE2-OP, which integrate conventional risk factors including age, sex, blood pressure, and cholesterol. However, these models do not capture the full biological and subclinical heterogeneity of CVD. Evidence suggests that imaging measures of atherosclerosis and genetic susceptibility, quantified by PRS [9], have the potential to substantially improve individual risk characterisation. Yet, whether this information enhance preventive behaviours or clinical outcomes remains uncertain.

The CVRISK-IT trial is designed to address this evidence gap by testing whether integrating imaging and/or genetic information into CVD risk communication produces meaningful changes in estimated risk, preventive behaviours, and long-term outcomes. By combining a large-scale, randomised design with a national infrastructure, the study aims to provide robust evidence on the clinical utility of precision-enhanced prevention strategies.

The trial leverages two validated and complementary risk modifiers: imaging to quantify subclinical atherosclerosis, and PRS to estimate inherited CVD susceptibility. Together, these tools bridge phenotypic and genetic determinants of risk, supporting a more comprehensive and personalised approach to prevention. The factorial randomised design allows assessment

of both the independent and combined effects of imaging and genetic information, providing insight into the potential synergistic benefit of multimodal risk communication.

Furthermore, the study uses of a dedicated digital platform, "MyCardioSpace",[21] to deliver results, monitor engagement, and support lifestyle counselling. During follow-up this platform enables continuous participant interaction through personalised recommendations (e.g., digital notifications and phone calls),remote data collection, ensuring consistency and active communication while improving trial efficiency. Such a hybrid digital–clinical model aligns with current trends in CVD prevention and may serve as a template for future large-scale studies. Samples form CVRISK-IT will also contribute to the BBDCARDIO biobank and the Italian Cardiology Network data infrastructure providing lasting value beyond the trial itself, and supporting future research on the determinants of CVD (e.g., lipoprotein(a), C-reactive protein) and other chronic conditions in collaboration with other Italian initiatives.[20]

If successful, CVRISK-IT could have substantial implications for both clinical practice and public health policy. Demonstrating that precision tools such as imaging and PRS improve adherence, treatment uptake, or estimated CVD risk would provide strong justification for their integration into European and national prevention guidelines. Furthermore, evidence from a large, real-world population will inform cost-effectiveness analyses, supporting decisions on resource allocation in preventive cardiology. The study also has the potential to refine risk communication strategies. Understanding how individuals respond to imaging and genetic information—emotionally, cognitively, and behaviourally—will provide insight into how personalised risk communication can most effectively promote lasting behavioural change.

Several potential limitations must be acknowledged. The large multicentre structure, involving 17 IRCCS institutes and affiliated centres, introduces potential heterogeneity in participant recruitment, data collection, and clinical practice. Although harmonised protocols and central quality control procedures have been implemented, minor inter-centre variability is inevitable. Imaging assessments represent another potential source of variation. Despite calibration, standardisation, and central review, differences in scanner technology and operator expertise may influence measurement precision. The genetic component, being fully centralised, is less susceptible to such variability. Risk communication sessions, which may be performed either in person or remotely, could vary in delivery style and patient engagement. While this pragmatic flexibility reflects real-world practice, it may introduce heterogeneity in risk perception and behavioural responses. Additionally, the requirement for digital access and literacy may lead to a study population that is not fully representative of the broader

population, potentially limiting external validity. Maintaining participant engagement and complete data linkage over extended follow-up periods will be essential to ensuring the reliability of long-term outcomes. Finally, extended long-term follow-up is envisioned to complement registry-based outcome tracking.

In conclusion, CVRISK-IT represents a major national initiative to evaluate whether imaging and genetic information can enhance CVD risk assessment and prevention in apparently healthy adults. Its combination of rigorous trial design, digital engagement infrastructure, and large-scale biobanking positions it to provide robust evidence on the clinical and behavioural impact of precision-based risk communication. By contributing to the Italian Cardiology Network and BBDCARDIO, the trial will help clarify the potential value of multimodal risk information for CVD prevention and provide a basis for future research on early detection and personalised health strategies across chronic diseases.

Funding:

This study was supported by Ricerca Corrente Reti funding (RCR-2023-23684267) from Italian Ministry of Health to IRCCS Policlinico San Donato.

Acknowledgements:

Management Team Committee: Lorenzo Menicanti - Scientific Director and Principal Investigator, IRCCS Policlinico San Donato – Chief of Rete Cardiologica; Emanuele Di Angelantonio - Scientist –Co-PI, Human Technopole; Ambra Cerri - Chief Operating Officer Research, IRCCS Policlinico San Donato; Francesca Colazzo - General Secretary, Rete Cardiologica; Giorgia Masina - Data Protection Officer; Chiara Barberio, Project Manager, IRCCS Policlinico San Donato; Carmelina Chiarello, Grant Officer, IRCCS Policlinico San Donato; Alice Maria Capuzzo and Ignazio Frusciante, Financial Office, IRCCS Policlinico San Donato.

Steering Committee: Lorenzo Menicanti - Scientific Director and PI, IRCCS Policlinico San Donato – Chief of Rete Cardiologica; Emanuele Di Angelantonio - Scientist –Co-PI, Human Technopole; Ambra Cerri - Chief Operating Officer Research, IRCCS Policlinico San Donato; Roberto Latini and Maria Carla Roncaglioni, Istituto di Ricerche Farmacologiche Mario Negri IRCCS; Maria Benedetta Donati and Amalia De Curtis, Istituto Neurologico Mediterraneo NEUROMED; Rosanna Cardani, IRCCS Policlinico San Donato; Damiano Baldassarre, IRCCS Centro Cardiologico Monzino; Giovanna Liuzzo, IRCCS Fondazione Policlinico Universitario A. Gemelli; Eloisa Arbustini, IRCCS Fondazione Policlinico San Matteo; Giuseppe Ferrante, IRCCS

Istituto Clinico Humanitas; Gianfranco Parati, IRCCS Istituto Auxologico Italiano; Marta Rigoni and Paola Cornelia Muti, IRCCS Multimedica.

Independent Advisory Board: John Danesh, University of Cambridge UK; Frank Visseren, University of Medical Centre Utrecht; Eva Prescott, University of Copenhagen.

Data Management Team: Andrea Stoppini, Stefania Pazzi, Rares Fosteris, Paolo Mosconi, CBIM – Consorzio di Bioingegneria e Informatica Medica; Stephen Kaptoge, University of Cambridge; Mauro Amato, IRCCS Centro Cardiologico Monzino.

ICN BBDCARDIO: Principal Investigator: Maria Benedetta Donati, Amalia De Curtis, Istituto Neurologico Mediterraneo NEUROMED and Rosanna Cardani, IRCCS Policlinico San Donato; Co-PI: Laura Valentina Renna, IRCCS Policlinico San Donato; Sara Magnacca, Istituto Neurologico Mediterraneo NEUROMED.

Data Protection Office: Giorgia Masina, Maria Claudia Maggio.

Communication Team: Alfredo Pascali, Carlotta Alfieri, Massimo Boni, Simone Montonati, Paola Langella.

CVRISK-IT Trial Group: Federico Ambrogi, Sara Boveri, Massimo Piepoli, Serenella Castelvecchio, Lucia Ramputi, Francesca Maria Lombardo, Gianluigi Guida, Andrea Attanasio, Giandomenico Disabato, Rosanna Cardani, Valentina Milani, Marco Ranucci, Sebastian Guelfi, Damiano Baldassarre, Mauro Amato, Roberta Baetta, Gualtiero Colombo, Pablo Werba, Giulio Pompilio, Francesca Colazzo, Emanuele Battezzati, Sara Caratozzolo, Giulia Castellaneta, Sonia Eligini, Beatrice Frigerio, Alessio Ravani, Daniela Sansaro, Daniela Coggi, Eloisa Arbustini, Leandro Gentile, Alba Muzzi, ,Giovanna Liuzzo, Anna Severino, Ambra Pia Gavillucci, Ilaria Conte, Giuseppe Ferrante, Gianluigi Condorelli, Laura Papa, Olga Protic, Cinzia Giammarchi, Anna Rita Bonfigli, Manlio Cipriani, Floriana Tortomasi, Maria Teresa La Rovere, Monica Lorenzoni, Egidio Traversi, Andrea Passantino, Tiziana Bachetti, Valeria Broglia, Gianfranco Parati, Martino Pengo, Chiara Lioce, Elisa Marchesi, Roberto Latini, Maria Carla Roncaglioni, Marta Baviera, Jennifer Meessen, Maria Luisa Ojeda Fernandez, Lidia Staszewsky, Deborah Novelli, Greta Agostini, Marta Rigoni, Paola Cornelia Muti, Francesco Bandera, Francesca Triani, Monica Mancino, Veronica Romano, Maria Benedetta Donati, Amalia De Curtis, Luigi Frati, Maria Rosaria Persichillo, Licia Iacoviello, Daniele Carnevale, Stefano Carugo, Chiara Boni, Fabio Blandini, Laura Della Corte, Pietro Ameri, Letizia Carelli, Roberta Venè, Maddalena Altare, Paolo Camici, Virna Vittozzi, Ilaria Salvato, Sofia Garavaglia, Cristina Tresoldi, Massimo Volpe, Massimo Fini, Margot Bastianutti, Michela Goffredo, Carlo Cavaliere, Marianna Manzone, Anna D'Agostino.

Conflict of Interest:

All Authors declare that they have no financial or non-financial conflicts of interest related to this work.

Data Availability Statement:

The data underlying this article will be shared on reasonable request to the corresponding author.

References:

1. Global Health Estimates: Life expectancy and leading causes of death and disability.

2. Timmis A, Aboyans V, Vardas P *et al.* European Society of Cardiology: the 2023 Atlas of Cardiovascular Disease Statistics. *Eur Heart J* 2024;**45**:4019–62.

3. Luengo-Fernandez R, Walli-Attaei M, Gray A *et al.* Economic burden of cardiovascular diseases in the European Union: a population-based cost study. *Eur Heart J* 2023;**44**:4752–67.

4. Visseren FLJ, Mach F, Smulders YM *et al.* 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. *Eur Heart J* 2021;**42**:3227–337.

5. Conroy R. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. *Eur Heart J* 2003;**24**:987–1003.

6. SCORE2 working group and ESC Cardiovascular risk collaboration. SCORE2 risk prediction algorithms: new models to estimate 10-year risk of cardiovascular disease in Europe. *Eur Heart J* 2021;**42**:2439–54.

7. Van Daalen KR, Zhang D, Kaptoge S *et al.* Risk estimation for the primary prevention of cardiovascular disease: considerations for appropriate risk prediction model selection. *Lancet Glob Health* 2024;**12**:e1343–58.

8. Mach F, Koskinas KC, Roeters van Lennep JE *et al.* 2025 Focused Update of the 2019 ESC/EAS Guidelines for the management of dyslipidaemias: Developed by the task force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). *Eur Heart J* 2025:ehaf190.

9. Schunkert H, Di Angelantonio E, Inouye M *et al.* Clinical utility and implementation of polygenic risk scores for predicting cardiovascular disease: A clinical consensus statement of the ESC Council on Cardiovascular Genomics, the ESC Cardiovascular Risk Collaboration, and the European Association of Preventive Cardiology. *Eur Heart J* 2025;**46**:1372–83.

10. Vrints C, Andreotti F, Koskinas KC *et al.* 2024 ESC Guidelines for the management of chronic coronary syndromes: Developed by the task force for the management of chronic coronary syndromes of the European Society of Cardiology (ESC) Endorsed by the European Association for Cardio-Thoracic Surgery (EACTS). *Eur Heart J* 2024;**45**:3415–537.

11. Elliott J, Bodinier B, Bond TA *et al.* Predictive Accuracy of a Polygenic Risk Score–Enhanced Prediction Model vs a Clinical Risk Score for Coronary Artery Disease. *JAMA* 2020;**323**:636–45.

12. Sun L, Pennells L, Kaptoge S *et al.* Polygenic risk scores in cardiovascular risk prediction: A cohort study and modelling analyses. *PLoS Med* 2021;**18**:e1003498.

13. Mosley JD, Gupta DK, Tan J *et al.* Predictive Accuracy of a Polygenic Risk Score Compared With a Clinical Risk Score for Incident Coronary Heart Disease. *JAMA* 2020;**323**:627–35.

14. Radford NB, DeFina LF, Barlow CE *et al.* Progression of CAC Score and Risk of Incident CVD. *JACC Cardiovasc Imaging* 2016;**9**:1420–9.

15. Coronary Artery Calcium and Polygenic Risk Scores For Cardiovascular Risk Prediction: Results From Two Population-Based Studies. *Am Coll Cardiol*.

16. Ihle-Hansen H, Vigen T, Berge T *et al.* Carotid Plaque Score for Stroke and Cardiovascular Risk Prediction in a Middle-Aged Cohort From the General Population. *J Am Heart Assoc* 2023;**12**:e030739.

17. Bao X, Xu B, Lind L *et al.* Carotid ultrasound and systematic coronary risk assessment 2 in the prediction of cardiovascular events. *Eur J Prev Cardiol* 2023;**30**:1007–14.

18. Naqvi TZ, Chao C-J, Butterfield RJ *et al.* Effect of Detection of Subclinical Atherosclerosis on Carotid Ultrasound on Cardiovascular Events in a Primary Prevention Clinical Setting. *J Am Soc Echocardiogr Off Publ Am Soc Echocardiogr* 2025;**38**:902–15.

19. SPIRIT-CONSORT. https://www.consort-spirit.org/.

20. Baldassarre D, Iacoviello L, Baetta R *et al.* Rationale and design of the CV-PREVITAL study: an Italian multiple cohort randomised controlled trial investigating innovative digital strategies in primary cardiovascular prevention. *BMJ Open* 2023;**13**:e072040.

21. MyCardioSpace. https://mycardiospace.cbim.it/login.

22. Di Castelnuovo A, Bonaccio M, Costanzo S *et al.* The Moli-sani risk score, a new algorithm for measuring the global impact of modifiable cardiovascular risk factors. *Int J Cardiol* 2023;**389**:131228.

23. Harris PA, Taylor R, Thielke R *et al.* Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009;**42**:377–81.

24. Harris PA, Taylor R, Minor BL *et al.* The REDCap consortium: Building an international community of software platform partners. *J Biomed Inform* 2019;**95**:103208.

25. Sauerbrei W, Abrahamowicz M, Altman DG *et al.* STRengthening Analytical Thinking for Observational Studies: the STRATOS initiative. *Stat Med* 2014;**33**:5413–32.

List of Figures:

Figure 1: Geographic distribution of the enrolling centres involved in the CVRISK-IT trial. Red dots: main recruiting centres (IRCCS); Pins: current affiliated centres (being updated)

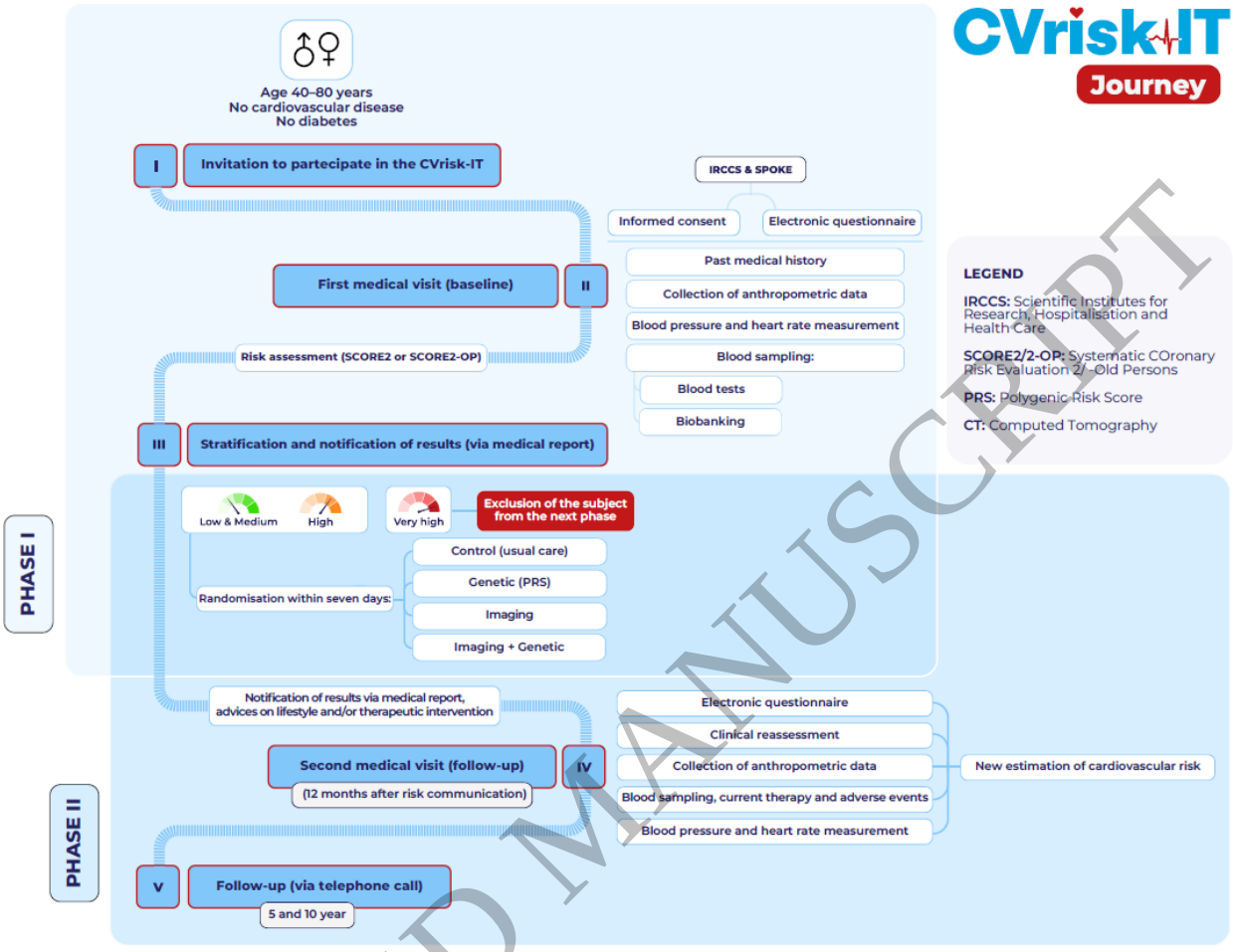


Figure 2: Study design and flowchart of the CVRISK-IT trial.

CARDIOVASCULAR RISK	< 50 YEARS	50-69 YEARS	≥ 70 YEARS
Low-to-moderate CVD risk: risk factor treatment generally not recommended	<2.5%	<5%	<7.5 %
High CVD risk: risk factor treatment should be considered	2.5 to <7.5%	5 to <10%	7.5 to <15%
Very high CVD risk: risk factor treatment generally recommended	≥7.5%	≥10%	≥15%

Table 1. 10-year CVD risk categories based on SCORE2 and SCORE2-OP in apparently healthy people stratified by age group. *Adapted from* [4]

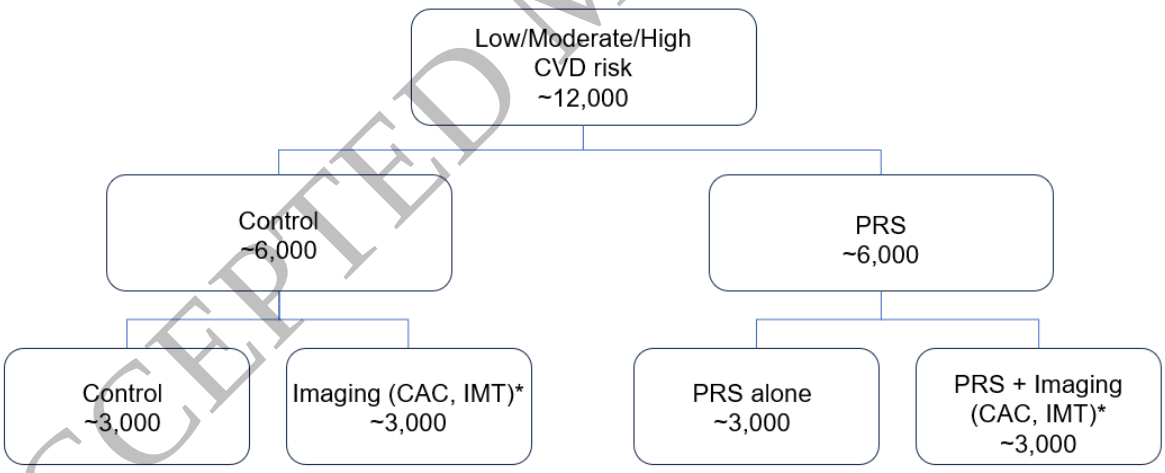


Figure 3. Factorial 2x2 randomisation design used in the CVRISK-IT trial. *Individuals are further randomised to CAC scoring or carotid ultrasound (IMT) based on centres feasibility.

Supplementary Material

Integrating genetic and imaging information to enhance cardiovascular risk stratification: rationale and design of the CVRISK-IT randomised controlled trial

Emanuele Di Angelantonio[1,2,3,4,5], Massimo Piepoli[6]*, Serenella Castelvecchio[7], Chiara Barberio[8], Carmelina Chiarello[8], Ambra Cerri[8], Sara Boveri[8], Rosanna Cardani[9], Federico Ambrogi[8, 10], Sebastian Guelfi[8,1], Damiano Baldassarre[11,12], Gualtiero I. Colombo[11], Mauro Amato[11], Roberta Baetta[11], Eloisa Arbustini[13], Giovanna Liuzzo[14,15], Stephen Kaptoge[2,3], Anna Severino[14,15], Giuseppe Ferrante[16,17], Maria Teresa La Rovere[18], Gianfranco Parati[19,20], Martino F. Pengo[19,20], Roberto Latini[21], Maria Carla Roncaglioni[21], Marta Rigoni[22,23], Amalia De Curtis[24], Luigi Frati[24],Pietro Ameri[25], Paolo Camici[26], Massimo Volpe[27], Lorenzo Menicanti[7,8]

eTable 1. List of enrolling sites*

IRCCS
1. IRCCS Policlinico San Donato (Milan)
2. Centro Cardiologico Monzino IRCCS (Milan)
3. Fondazione IRCCS Policlinico San Matteo (Pavia)
4. IRCCS Fondazione Policlinico Universitario A. Gemelli (Rome)
5. IRCCS Istituto Clinico Humanitas (Milan)
6. IRCCS Istituto Nazionale di Riposo e Cure per Anziani – INRCA (Ancona)
7. IRCCS Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione – ISMETT (Palermo)
8. IRCCS Istituti Clinici Scientifici Maugeri (Pavia)
9. IRCCS Istituto Auxologico Italiano (Milan)
10. Istituto di Ricerche Farmacologiche Mario Negri IRCCS (Milan)
11. IRCCS MultiMedica (Milan)
12. IRCCS Istituto Neurologico Mediterraneo Neuromed (Pozzilli)
13. IRCCS Fondazione Cà Granda – Ospedale Maggiore Policlinico (Milan)
14. IRCCS Ospedale Policlinico San Martino (Genoa)
15. IRCCS Ospedale San Raffaele (Milan)
16. IRCCS San Raffaele (Rome)
17. IRCCS Synlab SDN (Naples)
Affiliated centres
18. Azienda Ospedaliero Universitaria/Università di Napoli Federico II (Naples)
19. Azienda Ospedaliero Universitaria Mater Domini/Università Magna Grecia (Catanzaro)
20. Ospedale San Carlo Potenza (Potenza)
21. Ospedale Koelliker Torino (Turin)
22. Azienda Ospedaliero Universitaria di Pisa (AOUP), (Pisa)
23. IRCCS Ospedale Galeazzi – Sant'Ambrogio (Milan)
24. Azienda Ospedaliera S. Maria della Misericordia di Perugia (Perugia)
25. Ospedale S. Maria di Terni (Terni)
26. Azienda Ospedaliera "Cannizzaro" (Catania)
27. Fondazione Don Carlo Gnocchi (Parma)
28. Azienda Ospedaliero-Universitaria Integrata Verona (Verona)
29. Clinica Montevergine SpA, GVM Care and Research (Avellino)
30. Istituto di Medicina Aerospaziale dell'Aeronautica Militare "A. Mosso" Linate (Milan)
31. Medici di Medicina Generale di Via Pomponazzi 9 (Milan)
32. Centro di Cultura Socio-Sanitaria Pieve Emanuele (Milan)
33. Donatori di sangue: ASST-Brianza (Milan)
34. Clinica Polispecialistica San Carlo di Paderno Dugnano (Milan)
35. Associazione Avicenna (Palermo)
36. Associazione laboratorio ZEN (Palermo)
37. Azienda Ospedaliera "Ospedali Riuniti Villa Sofia – Cervello (Palermo)
38. ARNAS Ospedali Civico Di Cristina (Palermo)
39. Fondazione Istituto G. Giglio (Palermo)
40. Casa di Cura "Candela" (Palermo)
41. Azienda Ospedaliera ASST di Lecco (Lecco)
42. A.O.U. Policlinico "G. Rodolico- San Marco" (Catania)
43. Department of Radiology, Azienda Ospedaliero Universitaria (A.O.U.) (Cagliari)
44. Casa di Cura San Raffaele Sulmona (Sulmona, Aquila)

*List of affiliated centres in November 2025. List of enrolling IRCCS can be found here: https://www.cvrisk.it/cvrisk/scopri-lo-studio-cvrisk-it/centri-partecipanti;

eTable 2. Participant Timeline according to the SPIRIT Recommendations [1]

	Evaluation	Enrolment step (T0)	T1 (Month 0-6)	T2 (Month12) *	T3-4 (Year 5-10)
Phase I	Volunteers' invitation to participate Data privacy treatment and informed consent Biobank consent Inclusion/Exclusion	X			
	Electronic Questionnaire: -Age & Gender -Ethnicity -Marital Status -Previous medical /surgical history -Smoking habits -Diet habits -Current medical issues -Concomitant medication -Physical activity -Mental health assessment -Socio-economic factors	X		X	
	Clinical Examination: -Blood pressure (measured 3 times with 1-2 minutes interval in-between) -Body parameters -Vital signs (ex. cardiac frequency)	x		x	
	Collection of blood sample: -Total blood count -Total cholesterol -HDL cholesterol -Blood glucose -LDL cholesterol (calculated) -Triglycerides -Uricemia -Albumin	x		x	

	Point of care testing for cholesterol (POCT) – where applicable	x		x	
	Collection and storage of biological samples (e.g., biobanking)	x			
	Risk assessment using prediction models, such as SCORE2/SCORE-OP	x		x	
	Stratification according to predicted CVD risk: low-to-moderate risk, high risk, very high risk	x		x	
Phase II	Randomization into trial arms: -Control -Genetic (PRS) -Imaging -Imaging + PRS		x		
	Imaging-based risk assessment (B-mode ultrasound, cardiac computed tomography)		x		
	Genetic-based risk assessment - Polygenic Risk Score (PRS)		x		
	CVD prevention drugs treatment/compliance assessment		X	X	
	Tailored lifestyle advice to all participants		X	X	
	Kidney Organ damage assessment			x	
	Onset recording of cardiovascular-driven events			X	X
	Development and usage of a dedicated digital data platform	X	X	X	
	Follow-up through phone call with specialist and linkage with healthcare institution records				X

* (only for participants accessing Phase II)

eTable 3. Inclusion and Exclusion Criteria

Inclusion Criteria
Age: 40-80 years
Apparently healthy individuals without previous medical history of CVD or diabetes (based on medical diagnosis)
Participants must have signed the Informed Consent and Data Privacy Treatment forms
Participants must have a personal e-mail address and internet connection
Exclusion Criteria
Age <40 or >80 years
Individuals have previous medical history related to CVD (e.g., heart failure, congenital heart disorder, coronary heart disease) based on medical diagnosis
Previous history of type 1 or type 2 diabetes (based on medical diagnosis)
Medical diagnosis of mental disorders
Pregnancy based on self-reported information
Oncology patients (based on physician indication)
Candidates have previously participated in clinical studies of Italian Cardiology Network

Appendix A. Baseline visit procedures

The baseline visit represents the initial assessment of all participants enrolled in Phase I of the CVRISK-IT study. It includes the completion of informed consent procedures, lifestyle and medical history documentation, clinical examination, and biological sample collection for both immediate analyses and biobanking purposes. All activities are conducted in accordance with Good Clinical Practice and standard operating procedures shared across participating centres.

1. Informed Consent and Data Protection
Each participant provides written informed consent to participate in the study, as well as to the collection and storage of biological specimens for future research within the biobank of the Italian Cardiology Network BBDCARDIO. A separate personal data agreement form is also signed to ensure compliance with EU General Data Protection Regulation (GDPR). Documents are made available for online preview prior to the visit, while the official signature is obtained in person during the baseline appointment.

2. Questionnaire Administration
Participants complete a structured electronic questionnaire, either remotely before the visit or on-site using a tablet provided by staff. The questionnaire captures detailed information on:

•Demographic characteristics (age, sex, ethnicity, marital status, educational level, and socioeconomic indicators);
•Medical and family history, including previous cardiovascular conditions and relevant comorbidities;
•Lifestyle factors such as smoking status, alcohol consumption, dietary habits, and physical activity;
•Medication use, self-reported adherence, and preventive care history;
•Psychosocial and behavioural aspects, including mental health status and sleep quality.

The digital format ensures data completeness and consistency through automated validation checks.

3. Clinical Examination
A standardised physical examination is performed by trained personnel according to harmonised protocols across centres. Assessments include:

•Measurement of blood pressure in triplicate after at least 5 minutes of rest, following the recommendations of the 2023 European Society of Hypertension Guidelines for the Management of Arterial Hypertension [2];
•Recording of anthropometric parameters (height, weight, body mass index, waist and hip circumference) and vital signs (heart rate,);

4. Blood Sample Collection and Processing
Biological samples are collected for immediate testing and long-term storage:

• A capillary (using Point-of-Care Testing) or venous sample may be used for rapid assessment of lipids and glucose, enabling SCORE2 or SCORE2-OP risk estimation at the visit.
•Venous Blood Sampling: Five venous blood tubes (3X4mL and 2X5mL) are collected for laboratory testing and biobanking. Analyses include complete blood count, fasting glucose, lipid profile, and additional biomarkers as defined in the study protocol.

Samples for biobanking are processed within 2–4 hours of collection according to the Standard Operating Procedure of the biobank. Whole blood and plasma and serum fractions are aliquoted under sterile conditions in QR Code cryotubes and stored at −80 °C. All procedures are conducted according to standardized and validated protocols to ensure full traceability, optimal sample integrity, and long-term usability for research purposes.

Appendix B. Methods for cardiovascular risk reclassification

B1. *Low-dose non-contrast computed tomography for coronary artery calcium*

In participants assigned to the imaging group, coronary artery calcium (CAC) scoring is performed at baseline using low-dose, non-contrast cardiac computed tomography (CT), following international standards for image acquisition and quantification. The primary metric used for coronary calcification quantification is the Agatston score [3], with calcium volume and mass scores computed as secondary measures. The Agatston method quantifies the burden of coronary calcification as a weighted sum of all lesions with attenuation values exceeding 130 Hounsfield Units (HU). Each lesion's area (in mm^2) is multiplied by a weighting factor corresponding to its maximum attenuation: factor 1 (130-199 HU); factor 2 (200-299 HU); factor 3 (300-399 HU); and factor 4 (≥ 400 HU). The total Agatston score is obtained by summing the weighted lesion areas across all coronary arteries. In parallel, calcium volume score is determined by multiplying the number of voxels with attenuation > 130 HU by the individual voxel volume, while calcium mass is estimated using calibration phantoms for density correction. Participants are classified into four predefined CAC categories based on Agatston score thresholds [4]: 0, no detectable coronary calcification; 1–99, mild calcification; 100–299, moderate calcification; and ≥300, severe calcification.

Participants originally classified by the SCORE2/SCORE2-Older Persons (SCORE2-OP) algorithms as low-to-moderate or high risk will undergo reclassification according to their CAC score:

Original risk (estimated with SCORE2/SCORE2-OP)		**Risk modifier (CAC-Score)**		**Reclassified risk**
Low-to-moderate	+	0	→	Low-to-moderate
		1-299	→	High
		≥300	→	Very High
High	+	0-99	→	High
		≥100	→	Very High

B2. *Carotid ultrasound*

The presence and extent of subclinical atherosclerosis in the extracranial carotid arteries are evaluated at baseline by B-mode ultrasound using 10-13 MHz ultrasound transducers. All examinations are performed by trained sonographers according to a harmonised protocol to ensure reproducibility across centres. Quantitative carotid assessment includes measurement of the intima–media thickness (IMT) in the far wall of four arterial segments bilaterally: 1) the first centimetre of the internal carotid artery (ICA) proximal to bifurcation, 2) the bifurcation (Bif), 3) the first centimetre of the common carotid (1stCC) artery proximal to bifurcation, 4) the second centimetre of the common carotid (2ndCC) artery proximal to bifurcation. Each carotid artery (right and left) is scanned from three angles—lateral, anterior, and posterior—resulting in a total of 24 B-mode images per participant (4 segments × 3 angles × 2 sides). During the examination, images are acquired at end-diastole and stored digitally for central quality control. Measurements are performed in real time using the integrated calliper tools of the ultrasound system or, when available, through validated semi-automated analysis software (e.g., M'Ath, MIA).

For each segment, both mean and maximum (IMTmax) values are recorded. Mean IMT reflects the average arterial wall thickness and is considered a marker of diffuse arterial wall remodelling (e.g., smooth muscle hypertrophy or hyperplasia). Maximal IMT (IMTmax) identifies focal thickening consistent with early atherosclerotic change. A maximal IMT ≥ 1.5 mm is conventionally considered diagnostic of an atherosclerotic plaque. The IMTmax used for risk reclassification is defined as the highest of the four maximal IMT values measured across the left and right carotid arteries (1stCC-IMTmax, 2ndCC-IMTmax, Bif-IMTmax, ICA-IMTmax). Sex-

and age-specific best threshold values (BTVs), above which IMTmax is considered abnormally high, are applied according to previously validated population data [4]. In addition, the presence of hemodynamically significant carotid stenosis is recorded. Based on the North American Symptomatic Carotid Endarterectomy Trial (NASCET) criteria [5], stenosis is classified as severe when the NASCET ratio exceeds 70%.

According to 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice, a carotid plaque is defined either as the presence of a focal wall thickening that is ≥50% greater than the surrounding vessel wall, or as a focal region with an IMT measurement ≥1.5 mm that protrudes into the lumen. However, fixed cut-offs (e.g., 1.5 mm) may overestimate risk in older individuals, since IMT physiologically increases with age. Therefore, sex- and age-specific thresholds are applied in CVRISK-IT to better reflect the presence of pathologically elevated IMT values associated with vascular risk [6].

Participants originally classified by the SCORE2/SCORE2-OP algorithms as low-to-moderate or high risk will undergo reclassification according to carotid ultrasound cut-off:

Original risk (estimated with SCORE2/SCORE2-OP)		**Risk modifier: (age/sex IMT best cut-off)**		**Reclassified risk**
Low-to-moderate	+	IMT < best cut-off	→	Low-to-moderate
		IMT ≥ best cut-off	→	High
		Carotid stenosis > 70%	→	Very High
High	+	IMT < best cut-off	→	High
		IMT ≥ best cut-off	→	Very High
		Carotid stenosis > 70%	→	

B3. *Polygenic risk score*

Participants allocated to the genetic arm of the CVRISK-IT trial will receive comprehensive pre-test counselling to ensure informed decision-making and understanding of the genetic component of the study. During the counselling, participants will be fully informed about the meaning and implications of the PRS for their health. Counselling is conducted by trained medical personnel and includes detailed information on the purpose, scope, and methodology of polygenic risk testing, the quality standards applied to analytical procedures, and the process of result disclosure. In particular, participants will:

•Be informed that the PRS result is not diagnostic of any disease or health condition but represents a probabilistic estimate of an individual's predisposition to future cardiovascular disease relative to the general population

•Receive an explanation of the limitations of PRS testing, including the influence of ancestry, environment, and lifestyle;

•Be informed that replicate testing or additional sequencing analyses targeting other heritable traits are not planned within this project.

Genome-wide genotyping will be performed by a certified external laboratory. Genomic DNA will be extracted from peripheral blood using semi-automated protocols ensuring optimal yield, purity, and integrity. Genome-wide genotyping will be performed using high-density single-nucleotide polymorphism (SNP) arrays optimised for high imputation accuracy through imputation-driven marker selection (Illumina Infinium Global Screening Array v4.0). Genotyped data will undergo comprehensive quality control at both the SNP and individual levels, with SNPs filtered for Hardy-Weinberg Equilibrium (HWE p-value < 1×10^{-5}) and minimum genotyping call rate ≥ 98%. Individuals with genotyping rates < 95% are excluded and those with discordant reported versus genetic sex flagged for further inspection. Prior to imputation, Will Rayner's 1000G checking tool (https://www.chg.ox.ac.uk/~wrayner/tools/, version 4.2.13) validates

input data and corrects errors including inconsistent REF/ALT designations, strand misalignment, allele frequency deviations, and problematic palindromic SNPs. Imputation is performed using the Imputation Server 2 workflow on high-performance computing cluster with the 1000 Genomes Project Phase 3 reference panel (version 5).[7] Briefly, the workflow validates input data, phases haplotypes using the Eagle software and performs imputation with Minimac4. Finally, imputed variants are filtered to retain only those with imputation quality score (r^2) > 0.4 for subsequent PRS calculation.

Separate PRS are calculated for coronary heart disease (CHD) and ischemic stroke (IS) using the PGS Catalog Calculator tool (https://www.pgscatalog.org),[8] with the best-performing published GWAS selected for each disease based on independent cohort validation, sample size and ancestry composition matching the trial cohort. Effect-size-weighted SNP sums are computed and adjusted for ancestry to account for population structure. CHD and IS PRS are then combined into a single integrated score weighted by disease-specific incidence rates for the Italian population. PRS percentile thresholds are determined by calculating PRS in an Italian-specific reference cohort and mapping individual trial participants to these pre-defined percentiles. A final report is generated for each trial participant summarizing PRS risk stratification and interpretation guidance facilitate physician communication with patients.

The PRSs will classify individuals into two main risk categories (*i.e.*: high risk, PRS percentile >80%; low-to-intermediate risk, 0%-80%). A PRS above the 80th percentile is considered high because it confers on average more than 2-fold increased risk compared with the rest of the population. Notably, a PRS above the 95th percentile is considered very high because it confers on average more than 3-fold increased risk compared with the general population.

Participants initially classified by SCORE2/SCORE2-OP algorithms in the low-to-moderate or high-risk categories will be re-classified to his/her PRS percentile:

Original risk (estimated with SCORE2/SCORE2-OP)		**Risk modifier (*PRS percentile*)**		**Reclassified risk**
Low-to-moderate	+	0 – 80	→	Low-to-moderate
		80 – 95	→	High
		>95	→	Very High
High	+	0 – 80	→	High
		80 – 95	→	Very High
		>95	→	

B4. Combined PRS and Imaging-Based Cardiovascular Risk Reclassification

Participants who undergo both PRS assessment and imaging-based evaluation will have their cardiovascular risk category updated according to the most adverse (highest-risk) classification derived from either method. Specifically, individuals initially stratified by the SCORE2/SCORE2-OP algorithms as low-to-moderate or high risk will be reclassified using the following integrated approach as:

"Redefined" risk (obtained from <u>Imaging results</u>)		**Risk modifier (*PRS percentile*)**		**Reclassified risk**
Low-to-moderate	+	0 – 80	→	Low-to-moderate
		80 – 95	→	High
		>95	→	Very High
High	+	0 – 80	→	High
		80 – 95	→	
		>95	→	Very High
Very High	+	0 – 80	→	Very High
		80 – 95	→	
		>95	→	

In this integrated framework, reclassification is driven by the worst (highest-risk) result among the available modifiers. For example, a participant with a high polygenic risk (PRS > 80th percentile) but normal imaging findings will be reclassified to high risk; conversely, a participant with moderate genetic risk but a CAC ≥ 300 or carotid stenosis > 70% will be reclassified to the very-high-risk category.

This combined risk approach reflects the additive and complementary nature of genetic susceptibility and subclinical atherosclerosis in determining long-term cardiovascular outcomes, consistent with the 2025 ESC consensus on multimodal risk assessment [9].

Appendix C. Biobanking Procedures and Data Governance

C1. *Biological Samples*
All biological materials collected within the CVRISK-IT study are pseudonymised and tracked through a structured multi-level coding system designed to guarantee full traceability while protecting participant confidentiality. Each specimen is assigned a Biobank Code, which integrates information about the participating centre, the study or research project, and the individual enrolment sequence number. The Biobank Code does not contain any direct personal identifiers such as the participant's name or surname.

Each aliquot is further identified by a Sample Code, that incorporates the Biobank Code together with the type of biological material. All aliquots are registered within the Biobank Information Management System (BIMS) using the QR Code printed on each cryotube. Upon scanning, the system automatically links the QR code to both the Sample Code and the corresponding Biobank Code, thereby maintaining a secure and traceable connection between each Participant and their biological specimens. This digital workflow ensures accurate sample identification, efficient inventory management, and full traceability throughout the processing and storage lifecycle, while preserving participant confidentiality through pseudonymisation.

The database linking the Biobank Code to the participant's identity is maintained on encrypted servers protected by password-controlled access. Permission to access this information is granted only to authorised personnel at each IRCCS centre, including designated investigators, the Biobank Manager, and the healthcare professionals directly involved in participant management. Researchers using the stored samples or the derived datasets will receive only pseudonymised information and will have no access to personal identifiers.
All biological samples and associated genomic data are processed and stored in controlled-access facilities that comply with ISO-certified quality and safety standards. Entry to these areas is restricted to authorised staff who are admitted through secure authentication systems such as electronic badges, personal identification tags, biometric recognition, or physical key access.
Sample processing, storage, and transportation are conducted in accordance with Good Laboratory Practice and Good Clinical Practice principles to preserve sample integrity and ensure data reliability. Storage conditions are continuously monitored, with samples maintained at −80 °C in temperature-controlled freezers equipped with alarm and back-up systems to prevent loss or degradation. All operations are recorded within the central data management system to maintain full traceability and quality control.

C2. *Data and sample retention policy*
The management and retention of personal and biological data comply with the provisions of the General Data Protection Regulation (GDPR) and the corresponding Italian legislation on privacy and biobanking. Personal data collected for study purposes will be retained for a period of ten years after the formal conclusion of the trial, after which all identifiable information will be deleted or rendered irreversibly anonymous.
Biological samples and pseudonymised data stored within the BBDCARDIO biobank platform will be preserved for twenty-five years from the date of collection. After this period, biological specimens will be destroyed according to institutional biosafety procedures, and all associated personal data will be permanently deleted or anonymised. Every phase of storage, anonymisation, and destruction will be documented and archived to ensure verifiability and regulatory compliance under the supervision of the Biobank Manager.

Appendix D. Statistical Analysis

Sample size calculation

The power of a 2x2 factorial vs. parallel group randomised trial design was assessed by simulation assuming: 12,000 participants randomised, different co-primary outcome event rates for the control group (2% to 10%), and relative risks of 0.85 for two interventions (A and B) with/without different magnitudes of multiplicative interactions in the factorial design:

(a) No multiplicative interaction (RR_{A*B} = 1.00)

(b) Moderate-to-large synergistic multiplicative interaction (RR_{A*B} = 0.90)

(c) Moderate-to-large antagonistic multiplicative interaction (RR_{A*B} = 1.10)

For each scenario 12,000 participants were allocated in 1:1:1:1 ratio to Control:A:B:A+B in the factorial design (i.e. 3000 per group) and in 1:1:1 ratio to Control:A:B in the parallel group design (i.e. 4000 per group). Outcome for each individual was then simulated assuming the event rates implied by the parameters for the allocated group, i.e.

Group 1 (Control) = Control event rate

Group 2 (A only) = RR_A*Control event rate

Group 3 (B only) = RR_B*Control event rate

Group 4 (A+B) = (RR_A*RR_B*RR_{A*B})* Control event rate

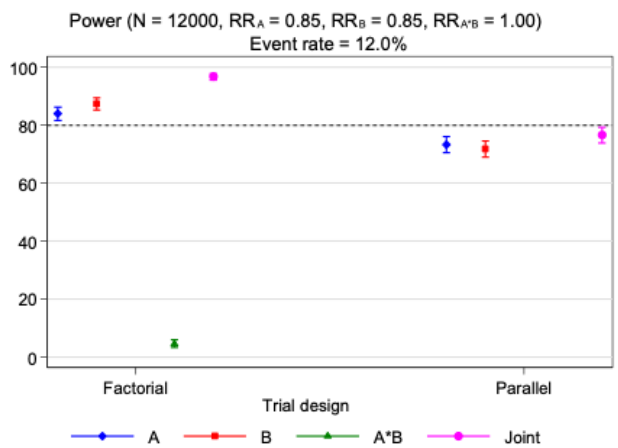
The simulated data were analysed with logistic regression model including the main effects of A and B, and in the factorial design also interaction of A and B (A*B). Resultant p-values were recorded for each parameter, and the simulations were repeated 1000 times for each scenario. The statistical power for each parameter is represented as the proportion of simulations with $p < 0.05$, which are displayed on the comparison plots.

Similar simulation approach was used for the secondary outcome, except assuming continuous outcome analysed by linear regression with effect sizes expressed additively in standard deviation (SD) units.

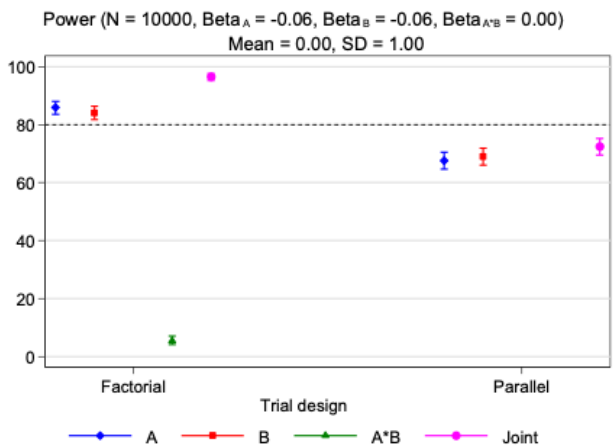
The procedure was coded in Stata software. The results indicated preference for a 2x2 factorial trial design over parallel group design, allowing simultaneous evaluation of two interventions with >80% power to detect the hypothesised intervention main effects.

Power calculation for co-primary outcomes in CVRisk-IT

Co-Primary outcome at long term follow-up



Co-Primary outcome at short term



Data analysis

The trial will be reported according to CONSORT guidelines for Factorial RCTs. Data curation and initial data analysis (IDA) will be performed according to the more recent guidelines of the STRATOS initiative [https://stratos-initiative.org/en/group_3]. A detailed statistical analysis plan will be drafted before any data is accessed.

The following steps are an integral part of IDA [10]:

I. Metadata setup aimed at systematically gathering all background information required to properly conduct all following IDA steps.

II. Data cleaning aimed at identifying and correcting errors in the data using the metadata for an efficient procedure.

III. Data screening consisting of understanding the properties and the quality of the data that may affect future analysis and interpretation.

IV. Initial data reporting aiming at informing all potential collaborators about all relevant insights obtained from the previous steps. It should provide all necessary information to properly conduct the intended analyses.

V. Refining and updating the analysis plan.

All the data will be checked using the frequency distribution to assess possible missing, errors or outliers. Descriptive statistics will be used to describe the sample characteristics, where categorical data will be presented as frequencies, and continuous data will be presented as means ± standard deviation (M±SD) for normally distributed variables and as the median and interquartile range (25°–75° percentile) for continuous non-normally distributed data. Visual inspection of histograms and QQ plots will be primarily used to judge appropriateness of normal distribution assumption, supplemented by calculation of skewness statistic or Kolmogorov–Smirnov test. Correlations between continuous variables will be evaluated according to Pearson R or Spearman Rho, depending on the distribution. Cluster analysis and visualization methods (Heatmap) will be used for exploratory data analysis.

Cluster analysis and visualization methods (Heatmap) will be used for exploratory data analysis.

Primary analyses will be based on the intention to treat (ITT) principle. Frequency of missing outcome data will be compared across intervention groups. Those with missing follow-up data will be excluded from the analyses (a complete case approach). We will perform sensitivity analyses to assess the impact of missing follow-up data on the results.

The effect of the study group (control group, genetic risk score group, imaging risk score group, and genetic + imaging risks score group) on CVD risk estimated using SCORE2 or SCORE2-OP at 12 months will be tested by two-way analysis of covariance (ANCOVA) evaluating the interventions main effects and interaction terms adjusted for centre and the baseline CVD risk. In presence of a statistically significant result for interaction term, estimates of the following pairwise differences will be calculated:

1) Group 2 (genetic risk score) vs. Group 4 (genetic + imaging risks score) – to estimate the effect of providing imaging risk information on top of genetic risk compared with the sole genetic risk;

2) Group 3 (imaging risk score) vs. Group 4 (genetic + imaging risks score) – to estimate the effect of providing genetic risk information on top of imaging risk compared with the sole imaging risk;

3) Group 1 (control group) vs. Group 4 (genetic + imaging risks score) – to estimate the effect of providing genetic risk information plus imaging risk information compared with no intervention.

In case of no significant interaction, estimates of the main effects will be provided estimating efficacy of genetic risk score and imaging risk score.

For pre-specified subgroup analyses of the co-primary endpoint, the analysis will be extended to include an interaction between the intervention effect with subgroup characteristics (e.g. age, sex). If the p-value for interaction is <0.01, then intervention effects will be calculated and reported separately by categories of the subgroup variables. For age and baseline CVD risk score, the cut-off for the categories will be their median and quartile values at baseline.

When long-term outcomes will be available (even if outside the study's funding period), overall CVD events rate and mortality will be analysed using the Kaplan-Meier method and the Cox regression model. The proportional hazards assumption will be verified using Schoenfeld residuals and formal test assessing interaction of interventions with follow up time.

In supplementary analyses CVD mortality or CVD events will be analysed accounting for death for other causes as competing event. A further competing risks analysis will be conducted on the first occurrence, during follow-up, of different types of CV events and death. The cumulative incidence function (CIF) will be used to assess the probability of CV events and death during the follow-up period. Gray's test will be used to evaluate hypotheses of no difference of crude cumulative incidence functions among the 4 trial arms.

Statistical analysis will be performed using SAS 9.4 (SAS Institute, Cary, NC) and R software. A 2-tailed P ≤0.05 will be considered statistically significant.

References

1. SPIRIT-CONSORT. https://www.consort-spirit.org/.

2. Mancia G, Kreutz R, Brunström M *et al.* 2023 ESH Guidelines for the management of arterial hypertension The Task Force for the management of arterial hypertension of the European Society of Hypertension: Endorsed by the International Society of Hypertension (ISH) and the European Renal Association (ERA). *J Hypertens* 2023;**41**:1874–2071.

3. Agatston AS, Janowitz WR, Hildner FJ *et al.* Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol* 1990;**15**:827–32.

4. Hecht HS, Blaha MJ, Kazerooni EA *et al.* CAC-DRS: Coronary Artery Calcium Data and Reporting System. An expert consensus document of the Society of Cardiovascular Computed Tomography (SCCT). *J Cardiovasc Comput Tomogr* 2018;**12**:185–91.

5. Bartlett ES, Walters TD, Symons SP *et al.* Quantification of carotid stenosis on CT angiography. *AJNR Am J Neuroradiol* 2006;**27**:13–9.

6. Baldassarre D, Amato M, Pustina L *et al.* Measurement of carotid artery intima-media thickness in dyslipidemic patients increases the power of traditional risk factors to predict cardiovascular events. *Atherosclerosis* 2007;**191**:403–8.

7. Das S, Forer L, Schönherr S, *et al*. Next-generation genotype imputation service and methods. *Nat Genet* 2016;**48**:1284-1287.

8. Lambert, Wingfield et al. (2024) Enhancing the Polygenic Score Catalog with tools for score calculation and ancestry normalization. *Nat Gen* 2024;**56**:1989-1994

9. Schunkert H, Di Angelantonio E, Inouye M *et al.* Clinical utility and implementation of polygenic risk scores for predicting cardiovascular disease: A clinical consensus statement of the ESC Council on Cardiovascular Genomics, the ESC Cardiovascular Risk Collaboration, and the European Association of Preventive Cardiology. *Eur Heart J* 2025;**46**:1372–83.

10. Huebner M, Le Cessie S, Schmidt CO *et al.* A Contemporary Conceptual Framework for Initial Data Analysis. *Obs Stud* 2018;**4**:171–92.